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Abstract

The precise distribution of thyrotropin releasing hormone (TRH) in 23 discrete brain nuclei and areas of Wistar strain male rats was determined by specific radioimmunoassay. TRH was detected in most of these areas. The highest concentration was found in the median eminence (27.52 +/- 2.84 ng/mg protein). The arcuate nucleus (4.92 +/- 0.58 ng/mg protein), dorsomedial nucleus (4.77 +/- 0.59 ng/mg protein) and medial preoptic area (3.94 +/- 0.15 ng/mg protein) also contained a considerable concentration of TRH. However, no TRH was detected in cerebral cortex, cerebellar hemisphere, anterior pituitary or pineal body. The data indicated that TRH was widely distributed throughout the hypothalamus; in particular, high concentrations occure in relatively restricted areas: in the median eminence, arcuate nucleus, dorsomedial nucleus and medial preoptic area. These areas coincide well with the so-called "thyrotropic" area of the hypothalamus.

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THYROTROPIN RELEASING HORMONE (TRH): ITS WIDESPREAD DISTRIBUTION IN DISCRETE HYPOTHALAMIC NUCLEI AND AREAS IN RAT BRAIN

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Abstract. The precise distribution of thyrotropin releasing hormone (TRH) in 23 discrete brain nuclei and areas of Wistar strain male rats was determined by specific radioimmunoassay. TRH was detected in most of these areas. The highest concentration was found in the median eminence (27.52±2.84ng/mg protein). The arcuate nucleus (4.92±0.58 ng/mg protein), dorsomedial nucleus (4.77±0.59ng/mg protein) and medial preoptic area (3.94±0.15ng/mg protein) also contained a considerable concentration of TRH. However, no TRH was detected in cerebral cortex, cerebellar hemisphere, anterior pituitary or pineal body. The data indicated that TRH was widely distributed throughout the hypothalamus; in particular, high concentrations occure in relatively restricted areas: in the median eminence, arcuate nucleus, dorsomedial nucleus and medial preoptic area. These areas coincide well with the so-called "thyrotropic" area of the hypothalamus.

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Since hypothalamic thyrotropin releasing hormone (TRH) was discovered in 1969 (1, 2), it has been shown to stimulate the release of thyrotropin (TSH) (1–3), prolactin (4–6) and growth hormone (GH) (6) by acting directly on the pituitary. Many papers have reported the effect of TRH on pituitary hormone secretion. However, there are only a limited number of studies concerning the precise distribution of this hormone in the hypothalamus and other brain areas. This problem has been approached by two main techniques: immunohistochemical method and nuclear punch method. Extensive studies with these techniques show that TRH is not exclusively in the hypothalamus, but is also present in extrahypothalamic brain regions (7–11). Only one report has appeared on the TRH concentrations in the discrete hypothalamic nuclei and areas of the rat by micropunch method (12). We developed an accurate selective micropunch method for various nuclei and areas of the rat brain by modification of Palkovits technique (13). At the same time, by this method, the TRH content and con-

centration in rat brain nuclei and areas can be determined by radioimmunoassay (RIA).

MATERIALS AND METHODS

Assay procedure of TRH. Radioimmunoassay of TRH was performed according to the methods of Utsumi *et al.* (14) with slight modification. Three hundred microliter of assay buffer containing 0.1% gelatin and 0.1M EDTA 2Na in 0.01M phosphate 0.15M saline (pH 7.6), 100 μl of sample or standard solution, 100 μl of ^{125}I-TRH and 100 μl of anti-TRH antiserum (1 : 12,000) were placed into the plastic tube. After 48 h incubation at 4°C, 100 μl of second antibody and 100 μl of diluted normal rabbit serum were added. All tubes incubated for an additional 24 h, centrifuged, the supernatant was decanted, and radioactivity in the precipitate was counted.

Removal of various hypothalamic regions and extraction of TRH. Mature Wistar strain male rats weighing about 200g were housed under controlled illumination (12 h of light starting at 7 a.m.) and given free access to food and water for ten days. After adaptation to the new environment, they were decapitated between 11 and 12 a.m. and whole brains were immediately removed. The brains were then stood caudal portion up, and frozen with powdered dry ice as quickly as possible. They were serially sectioned alternately at 100 μm for the histological placement and at 400 μm for further removal of the hypothalamic nuclei and areas between the rostral portion of the optic chiasma and the center of the mammillary body with a freezing-microtome (Komatsu Electric Inc., Model 201, Japan) at −20°C (Fig. 1).

One hundred micrometer sections were fixed with folmalin-ethanol solution (10% folumalin : ethanol=3 : 1), and were stained with toluidin blue. They were serially photographed to confirm the location of hypothalamic nuclei. These photographs served as references for the micropunch method.

Each 400 μm section was immediately mounted on conventional slide glass, frozen on dry ice again, and stocked at −60°C until removal of the hypothalamic nuclei and areas. The micropunch method of nuclei was performed with specially designed needles under a stereomicroscope according to the rat brain atlas (15). The inner diameter of the needles was from 200 μm to 1,100 μm. Twenty subdivisions were removed while keeping the sections frozen on dry ice. Anterior and posterior pituitaries and pineal bodies were also removed. Fig. 2 illustrates a section at the medial hypothalamus after removal of the intended areas.

The removed materials were immediately placed in 1 ml of an ice cold mixture of methanol and 1M acetie acid (4 : 1, v/v). The tissues were homogenized with a microhomogenizer (Wheaton Scientific Co. Teflon type 1 ml homogenizer) : Fifty to 200 μl of each homogenate was taken for protein determination (16). The remainder was centrifuged at 1,200×g for 30 min, the resulting supernatants being dried at 60°C under a continuous N_2 gas stream and then stocked at −20°C until TRH assay. The dried material was resuspended in the assay buffer for TRH as described above, and centrifuged again. TRH measurement was car-

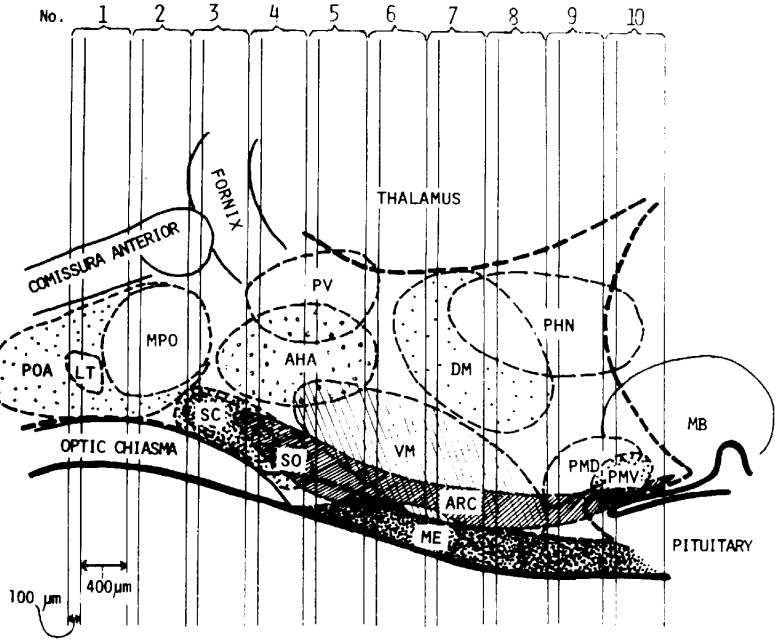

Fig. 1. Serial section pattern of the rat hypothalamus. Numbers shown at the top of the illustration indicate the section order from the rostral portion of the optic chiasma to the center of the mammillary body in the thickness of 100 μm and 400 μm alternately. Abbreviations: see Table 1.

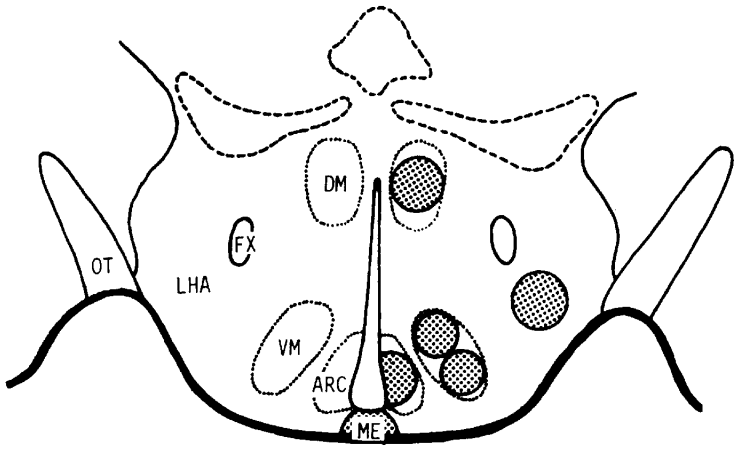

Fig. 2. Schematic section at the medial hypothalamus. Shadows indicate the removed area.

ried out using triplicate samples of all extracts for RIA.

Recovery experiments were performed by the addition of a known amount

of TRH equivalent to endogeneous levels of aliquots to tissue extracts assayed with and without added hormone. This was done in triplicate for extracts of cerebral cortex. The mean recovery of added hormone was 88.0%.

The intraassay coefficient of variation for dose levels 50pg to 5,000pg was 6.4% mean. The interassay coefficient of variation at the same level was 10.4% mean. Assay sensitivity was 10pg per tube.

RESULTS

The TRH content and concentration in various part of the rat brain are given in Table 1. Immunoreactive TRH was detected in not only the hypo-

TABLE 1. TRH CONTENT AND CONCENTRATION IN DISCRETE RAT BRAIN AREAS

Brain Regions	Abbreviation	TRH	
		Concentration ng/mg protein	Content pg/nucleus[a]
1. Preoptic area	POA	0.86±0.11[b]	
Medial preoptic area	MPO	3.94±0.15	136± 16
Lamina terminalis	LT	1.71±0.22	86± 14
2. Hypothalamus			
Nucleus hypothalamicus anterior	AHN	2.23±0.28	258± 29
Nucleus suprachiasmaticus	SC	1.37±0.13	75± 7
Nucleus supraopticus	SO	1.06±0.26	38± 6
Nucleus paraventricularis	PV	3.08±0.32	219± 22
Lateral hypothalamic area	LHA	1.57±0.10	
Nucleus ventromedialis	VM	2.25±0.19	293± 35
Nucleus dorsomedialis	DM	4.77±0.59	510± 47
Nucleus arcuatus	ARC	4.92±0.58	421± 34
Nucleus hypothalamicus posterior	PHN	2.05±0.73	106± 20
Nucleus premammillaris dorsalis	PMD	1.90±0.38	85± 13
Nucleus premammillaris ventralis	PMV	2.19±0.26	137± 20
Median eminence	ME	27.52±2.84	912±121
3. Septum			
Nucleus lateralis	SL	1.76±0.26	
Nucleus medialis	SM	0.68±0.14	
4. Nucleus accumbens	ACB	1.14±0.35	
5. Cerebral cortex	CX	ud	
6. Cerebellar hemisphere	CH	ud	
7. Pituitary			
anterior	AP	ud	
posterior	PP	0.35±0.08	142± 39
8, Pineal body	PN	ud	

a: All the nuclei were not punched out completely. Therefore, the TRH content in each nuclei might be slightly greater than the levels in this Table.

b: Mean±SEM ud: undetectable Nine rats per group were used.

thalamus but throughout the extrahypothalamic brain regions. When expressed as ng per mg protein, the highest concentration of this hormone was found in the median eminence (27.52 ± 2.84 ng/mg protein), followed by the arcuate nucleus (4.92 ± 0.58), the dorsomedial nucleus (4.77 ± 0.59) and the medial preoptic area (3.94 ± 0.15). The paraventricular nucleus (3.08 ± 0.32), the ventromedial nucleus (2.25 ± 0.19) and the anterior hypothalamic nucleus (2.23 ± 0.28) also contained a considerable concentration of TRH. A small amount of this hormone was detected in the accumbent nucleus (1.14 ± 0.35) and in the posterior pituitary (0.35 ± 0.08) When expressed in terms of content per nucleus, a largest quantity of TRH was found in the median eminence (912 ± 121pg). The dorsomedial nucleus (510 ± 47pg) and the arcuate nucleus (421 ± 34pg) also contained a large amount of this peptide. Moderate contents of TRH were detected in the ventromedial nucleus (293 ± 35pg), the anterior hypothalamic nucleus (258 ± 29pg) and the paraventricular nucleus (219 ± 22pg). However, no TRH was detected in the cerebral cortex, cerebellar hemisphere, anterior pituiary or pineal body.

As can be seen in Fig. 3, high concentration of TRH occurred in relatively restricted areas: the median eminence, the arcuate nucleus, the dorsomedial nucleus and the medial preoptic area.

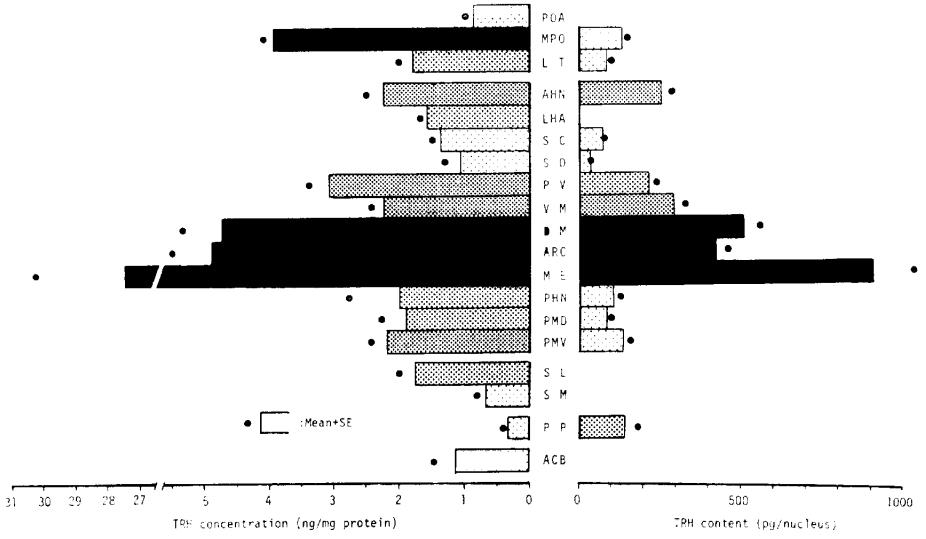

Fig. 3. Localization of TRH in hypothalamus, preoptic area, septum, posterior pituitary and accumbent nucleus. Darker bars indicate larger content and concentration of TRH. Abbreviations: see Table 1.

DISCUSSION

The present study shows that TRH is widely distributed throughout the hypothalamus; in particular, high concentrations of this hormone occure in relatively restricted areas: median eminence, arcuate nucleus, dorsomedial nucleus and medial preoptic area. These results do not agree with the earlier report of Brownstein *et al.* (12). The concentration of TRH in the median eminence in my report (27.52±2.84ng/mg protein) is similar to theirs (38.38± 8.27ng/mg protein), but the concentration and content in the ventromedial nucleus is about one fourth lower in the present report. The anterior hypothalamic nucleus, paraventricular nucleus and dorsal premammillary nucleus also contained a relatively large amount of TRH. A small amount of TRH was also detected in the accumbent nucleus which Hökfelt reported to contain a high density of TRH-positive fibers (9). The cause of the discrepancy between these results is unclear. It may be due to the differences in the sex or strain of rats used. Otherwise the difference of the handling stress or the time of the day which were reported to influence the hypothalamic TRH content (17-19).

Secretion of TSH is controlled by the thyroid hormone concentration in pituitary thyrotrophs so as to maintain a relatively constant level of thyroid hormone in blood. The feedback effects of thyroid hormone at the pituitary level are of major importance in the control of TSH secretion. Techniques used for studying neural control of anterior pituitary hormone also provide information on CNS concerned with TRH production and/or storage. The effects of brain lesions on the function of the thyroid were examined by Greer (20) who delineated a "thyrotropic" area of the brain in the midline between the paraventricular nucleus and the median eminence. Studies in which anterior pituitary tissue was transplanted into various regions of the hypothalamus showed that normal structure and function of the anterior lobe are maintained only in those animals in which the graft was situated in the so-called "hypophysiotropic" area (21-23). This area which consists of the arcuate nuclei, the ventral part of the anterior periventricular nuclei, the medial part of the retrochiasmatic area, and the median eminence was identified as the site of secretion and release of hypothalamic hormones.

Several attempts have been made to define the hypophysiotropic area of the hypothalamus by electrical stimulation. In the rat, radioimmunoassayable plasma TSH levels rose rapidly after stimulation of the hypothalamus or the anterior part of the median eminence (24). Martin and Reichlin reported identical results in detail (25). The area from which positive responses were obtained was widely distributed in the hypothalamus, including the preoptic area, paraventricular nucleus, anterior hypothalamic area and medial basal hypo-

thalamus. The largest responses in the rat occurred with stimulation of the anterior hypothalamus adjacent to the paraventricular nuclei. Negative responses occurred with stimulation of the lateral hypothalamus, thalamus and mammillary body. A similar distribution of effective stimulation sites for TSH release was found by Averill and Salaman in the rabbit using a TSH bioassay (26). The extensive field of positive stimulation sites corresponds well with the "hypophysiotropic" area of Halász (27). He employed a deafferentation technique using his knife to examine the brain-hypothalamo-pituitary interaction. In his report, a moderate decrease in levels of basal serum thyrotropin was followed by hypothalamic deafferentation. Therefore, before the preparation of the specific antibody to the TRH, the most important part for the maintenance of hypothalamo-hypophyseal-thyroid function in the brain area was from the preoptic area to the anterior part of the hypothalamus.

After isolation and determination of the structure of TRH (1, 2), synthetic TRH was produced and specific antibody made (28). Utilizing such antibody, immunohistochemical studies were performed by Hökfelt in various brain areas (9). TRH-containing nerve terminals were found in the dorsomedial nucleus and the perifornical area, in extrahypothalmic nuclei such as nucleus accumbens, the lateral septal nucleus and several motor nuclei of the brain stem and spinal cord. Also, a more direct approach for assessment of the topographic releasing factor content was used by Krulich *et al.* (29). In studies in which freshly frozen hypothalami were sectioned in three planes to determine the location of TRH by *in vitro* TSH assay, activity was found in the three restricted areas. The anatomical distribution of moderately high concentrations of TRH sites reported was also found in the present study: namely, the median eminence-arcuate nucleus area, dorsomedial nucleus and medial preoptic area. Therefore, the so called "thyrotropic" area contained a large amount of TRH.

However, unlike luteinizing hormone releasing hormone (LHRH), which is located in restricted areas of the rat brain at the median eminence, arcuate nucleus and circumventricular organs including the organon vasculosum of lamina terminalis (OVLT) (30–32), TRH is widely distributed throughout the hypothalamus and other brain areas as demonstrated by my present data and others (7–12). Therefore, it has been suggested, on the basis of its widespread distribution in the brain area and other pharmacological effects (33–35), that this hormone is not solely restricted to the hypothalamic neurons involved in pituitary hormone secretion, but that it may also serve as a neurotransmitter or modulator of neutronal activity in the brain.

The measurement of TRH levels in the hypothalamus might be of limited value for kinetic analysis of TRH synthesis and secretion. For more direct studies about the effects of thyroid status on TRH secretion, several workers

have utilized RIA of TRH in urine and blood (8, 14, 36). Interpretation of the results of these studies is difficult. TRH is found in the hypothalamus and in other parts of the central nervous system as well so that the significance of measurements of TRH in urine or blood is uncertain. It is not known whether TRH present in extrahypothamic sites is regulated in the same way as this peptide located in the hypothalamus itself. Moreover, the methods of assessing serum and urine TRH concentrations are not reliable at present, and there is no way to measure directly the rate of TRH turn-over. So, it would be worthwhile trying to analyze TRH concentrations in different parts of the brain area.

Therefore, it is necessary to examine the diurnal rhythm of this hormone in discrete hypothalamic nuclei and areas, and also, the changes of TRH time course under various stress conditions.

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